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(54) Responsive paramagnetic MRI contrast agents

(57) A method is claimed based on CEST procedure for the *in vivo* or *in vitro* determination of physico-chemical parameters which includes the use of a paramagnetic CEST contrast agent.

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Description

[0001] This invention refers to a method based on CEST procedure for the *in vivo* or *in vitro* determination of physico-chemical parameters of diagnostic interest comprising the use of at least one paramagnetic contrast agent and to the pharmaceutical composition for use therein.

BAKGROUND OF THE INVENTION

[0002] It is now well established that the potential of Magnetic Resonance Imaging (MRI) procedures can be further enhanced when this diagnostic modality is applied in conjunction with the administration of contrast agents (CAs), i.e. chemicals able to promote marked changes in the relaxation rates of the tissue protons. According to the major effects they produce on images, CAs are classified as positive or negative agents. The positive CAs are represented by paramagnetic complexes, mostly containing Gd(III) or Mn(II) ions, which affect the relaxation rates of the bulk water through the exchange of the water molecules in their coordination spheres (Caravan P, et al. Chem Rev 1999, 99: 2293-2352; the Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging. Chichester, UK: John Wiley & Sons; 2001. p 45-120). Their effect is similar on T_1 and T_2 but, being T_1 usually significantly longer than T_2 in most biological tissues, their effect is more often exploited in T_1-weighted images, thus resulting in brighter spots in the images.

[0003] On the contrary, negative CAs are used to shorten T_2, leading to an improved contrast by reducing the water signal in T_2-weighted images.

[0004] Furthermore, it was early reported that chemicals containing mobile protons may act as T_2-agents through the reduction of the water proton relaxation time *via* exchange processes (Aime S et al., Invest Radiol 1988; 23(Suppl 1):S267-S2706). A different approach in order to efficiently reduce the water signal has been shown to occur when a proper radiofrequency rf irradiating field is applied at the resonance frequency of the exchangeable protons saturating it. This results in a net decrease of the bulk water signal intensity owing to a saturation transfer effect.

[0005] Balaban and coworkers named this contrast-enhancing procedure Chemical Exchange Dependent Saturation Transfer (CEDST or, more commonly, CEST) (Balaban RS.: Young IR, editor. Methods in Biomedical Magnetic Resonance Imaging and Spectroscopy. Chichester, UK: John Wiley & Sons; 2000. Vol.1. p 661-6667).

[0006] Very recently, Sherry et al. showed that a particularly useful source of highly shifted exchangeable protons can be provided by the slowly exchanging water protons bound to a paramagnetic Eu(III)-chelate. In this complex, the irradiation of such protons, which resonate at ca. 50 ppm downfield from the bulk water signal, determined a significant CEST effect in the images obtained at 4.7 T. (Sherry et al. in J Am Chem Soc 2001, 123:1517-1518).

[0007] The efficacy of high majority of the prior art contrast agents, either the conventional T_1 and T_2-reducing MRI contrast agents, or the Eu(III)-based chelate CEST agents proposed by Sherry, is, however, related to the different cellular uptake of the administered complex compound or to the different distribution thereof trough the extracellular spaces of the targeted organ or tissue. No contrast is detectable if the uptake between the target and the surrounding tissue is similar.

[0008] Moreover, these contrast agents, in general, allow the production of images of the targeted tissue or organ but they all are unable to measure the metabolic conditions of the examined tissue and to refer about the physico-chemical parameters determining thereof.

[0009] In WO 00/66180, Balaban and co-workers disclose a method for obtaining an image by MRI which comprise the administration of a CEST MRI contrast agent containing chemical groups endowed with appropriate proton exchange and chemical shift properties to function effectively for performing CEST MRI analyses *in vivo*. Claimed method is said to be useful for determining certain physico-chemical parameters such as pH and temperature both *in vivo* and *in vitro*. The Application further discloses a ratiometric method for the pH measurement which is independent of the contrast agent concentration and which includes the administration of a CEDST contrast agent having two different exchangeable protons.

[0010] The agents disclosed by Balaban an co-workers as useful to practice the claimed method are diamagnetic organic molecules such as sugars, amino acids, nitrogen-containing heterocycles, purines, guanidine, nucleosides, imidazole and derivatives thereof, barbituric acid and analogous thereof, wherein heterocyclic compounds having exchangeable OH or NH groups such as 5,6- dihydrouracil, 5-hydroxytryptophan are particularly preferred when the pH is determined according to claimed method.

[0011] In general, the mobile protons of a contrast agent for a CEST application have to possess a fast exchange rate with water protons (k_{ex}), but slower than the coalescence condition, that is to say $k_{ex}\Delta\nu \sim 1/2\pi$, where $\Delta\nu$ is the chemical shift separation in Hz between the two exchanging pools. In this context, larger $\Delta\nu$ values enable the exploitation of higher k_{ex} values, thus resulting in an enhanced CEST effect.

[0012] The Balaban diamagnetic systems are advantageously endowed with short relaxation rates. Unfortunately, however, the chemical shifts of exchangeable protons thereof are only slightly shifted (~ 1-5 ppm) from bulk water

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signal and, therefore, slower exchange rate can be exploited before coalescence takes place.

[0013] Beside the small $\Delta\nu$ values, a further limit of such diamagnetic agents is due to the high concentration thereof which is usually required to generate a sufficiently large CEST effect wherein this results in an high probability of toxic or physiological effect *in vivo*.

SUMMARY OF THE INVENTION

[0014] The present invention relates to a method based on the CEST procedure for the *in vivo* or *in vitro or ex vivo* determination of a physico-chemical parameter of diagnostic interest which includes the administration of at least one paramagnetic compound.

[0015] In particular, it is an object of the present invention a method for the determination, by use of the Magnetic Resonance Imaging technique, of a physico-chemical parameter in a human or animal body organ, fluid or tissue wherein a CEST contrast agent is used the saturation transfer capability of which is correlated to the physico-chemical parameter of interest and a CEST MR image responsive for said parameter is registered, said method being characterized in that the CEST contrast agent comprises a paramagnetic complex compound.

[0016] The paramagnetic contrast agent for use in the method of the invention is a responsive agent, i.e. an agent which combines the characterising features of a CEST agent with the fact that the saturation transfer effect that it enables is sensitive to the physico-chemical parameter of diagnostic interest. Its use according to the method of the invention allows the registration of a CEST MR image which is responsive for said parameter in the organ or tissue under examination.

[0017] Accordingly, the contrast agent for use in the method of the invention is a paramagnetic compound which comprises at least one mobile proton in chemical exchange with the water medium protons and which is able, when a proper radiofrequency rf irradiating field is applied at the resonance frequency of the said exchangeable proton, to generate a saturation transfer (ST) effect between said mobile proton and the water medium protons wherein said saturation transfer correlates to the physico-chemical parameter of diagnostic interest.

[0018] In comparison with the CEST agents of the cited prior-art, the choice of a such paramagnetic complex compound offers the opportunity to suitably select and improve the basic requisites of a CEST-contrast agent. Accordingly, the molecular structure of the paramagnetic agents for use in the method of the invention can advantageously be selected in order to pursue optimal values for the chemical shifts and exchange rates of the mobile protons with water protons.

[0019] In comparison with the paramagnetic Eu(III) chelate proposed in J Am Chem Soc 2001, 123:1517-1518, a further advantage is represented by the possibility to irradiate a higher number of mobile protons different from those of the metal coordinated water protons, thus allowing to extend the CEST application also to the lanthanide complexes endowed with fast exchanging metal bound water or without any coordinated water molecule.

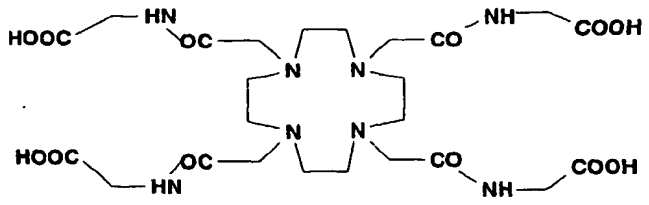
[0020] In comparison with the diamagnetic agents of WO 00/66180 which have structures including a relatively small number of mobile protons, the number of labile protons on the paramagnetic CEST agents according to this invention can advantageously be increased with consequent reduction of the administered dose. Further, when compared with the diamagnetic compounds, the large shift induced by the paramagnetic centre on the resonances of exchangeable protons increases their chemical shift separation from bulk water, thus allowing the exploitation of higher k_{ex} values.

[0021] The responsive paramagnetic agent for use in the method of the invention preferably includes at least one chelated complex of a paramagnetic metal ion. The paramagnetic metal ion is any transition or lanthanide (III) metal ion which has an electronic relaxation time suitably short to significantly affect the chemical shift value of the mobile protons to be irradiated. Preferred are paramagnetic metal ions selected in the group consisting of: iron (II) (high spin), iron (III), cobalt (II), copper (II), nickel (II), praseodymium(III), neodimium (III), dysprósium (III), erbium (III), terbium (III), holmium (III), thulium (III), ytterbium (III), and europium (III). Particularly preferred are lanthanide (III), also referred to as Ln(III), metal ions of this group. The chelating ligand of the paramagnetic complex for the use according to the invention can be any organic ligand endowed with at least one mobile proton bound to a nitrogen, oxygen, sulphur or phosphorous atom. Preferably the mobile proton belongs to an amide group coordinated to the metal ion.

[0022] A further suitable source of mobile protons according to the invention is represented by the water molecule (s) coordinated to the paramagnetic centre. In this particular case the relaxation time of the bulk water protons is influenced by the exchange thereof with the inner-sphere coordinated water protons.

[0023] Preferred paramagnetic complexes for use in the method of the invention are the chelates of the macrocyclic tetra-amide derivatives of the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) or of the tris-amide derivatives of the 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A) with the preferred metal ions indicated above.

[0024] Particularly preferred are the chelates of the 1,4,7,10-tetraazaciclododecane-1,4,7,10-acetic acid tetraglycineamide, herein referred to as DOTAM-Gly, of formula:



DOTAM-Glv

with said Ln(III) preferred paramagnetic ions.

[0025] These complexes possess two pools of exchangeable protons represented by the two protons of the water molecule coordinated to the metal centre and the four equivalent amide protons in the close proximity to the paramagnetic centre, respectively.

[0026] In comparison with DOTAM-Gly tetra ethyl ester (DOTAM-Gly-Et) disclosed in the cited prior-art, DOTAM-Gly provides a system endowed with one residual negative charge upon formation of the complexes with Ln(III) ions. These single negatively charged contrast compounds are expected to be better tolerated than those having three positive charges for which hydrolase activity on biological substrates has been documented (Epstein DM, Chappell LL,. Inorg Chem 2000; 39: 2130-2134).

[0027] The tetraglycineamide derivative of the DOTA, DOTAM-Gly, as well as its paramagnetic chelate complexes, particularly with Ln(III) metal ions and the physiologically acceptable salt thereof are new and constitute a further object of the present invention.

[0028] The CEST effect arising from the irradiation of the amide N-H protons of the Ln(III) complex compounds according to the invention has been tested on the basis of a series of CEST experiments in which the ST effect is measured as a function of the irradiation time (irradiation power of 1050 Hz). In particular five Ln-DOTAM-Gly chelates in which Ln = Dy, Ho, Er, Tm, Yb were tested. The experimentation results included in Figure 3 show that the most efficient saturation transfer is observed for the Yb(III) complex (30 mM) at pH 8.1 wherein the observed saturation effect of ca. 70 % after 2 s of irradiation appears indicative of a very efficient saturation transfer. To reach similar results with diamagnetic molecules, such as aminoacids, a much higher concentration (125 mM) is requested. Furthermore, similar results are obtained only at and pH 5, that is to say at pH values that are far from the physiological range (Ward KM et al. J Magn Res 2000; 143: 79-87).

[0029] As far as other Ln(III)-DOTAM-Gly complexes are concerned, the data reported in Figure 3 indicate a clear trend in the saturation transfer effect along the lanthanide series on passing from the Dy(III) chelate, for which the CEST effect is minimal, to the Yb(III) complex.

[0030] The overall visualization of the ST effects is represented by a CEST spectrum, an example of which is reported in Figure 4 for a 30 mM solution of Yb-DOTAM-Gly at pH 8.1. The spectrum reports the intensity of the water signal, normalized to the higher value, as a function of the irradiation offset. Obviously, the effect is maximum at the resonance frequency of water (0 ppm), but it is immediately evident that when the irradiation frequency is set to ca. -16 ppm from water, a significant water saturation is observed and the residual signal is slightly less than 20%. However, since this effect may be partly accompanied by the direct saturation of the water signal, the "true" CEST effect is quantitatively assessed by considering also the off-resonance saturation. This means that the value of the M_S/M_0 ratio measured in the experiment reported in Figure 3 is the ratio of the two values indicated by the arrows in the Figure 4.

[0031] For the Eu-DOTAM-Gly chelate the chemical shift separation between the amide protons and the bulk water is smaller (4.2 ppm) than the other Ln(III) ions tested (see Figure 2). The irradiation of the amide protons with the same square pulse used for the other Ln-DOTAM-Gly complexes does not allow the detection of a saturation transfer effect owing to a remarkable direct saturation effect on the bulk water. For this reason, it is convenient to use a selective shaped saturation pulse. The ST effect measured by using a train of 270° e-burp1 pulses of 20 ms each (total irradiation time 4s, irradiation power 50 Hz) was of 23% (pH 7.7, 30 mM, 312°K, 7.05T).

[0032] It is noteworthy to consider that in this chelate it is also possible to detect a saturation transfer effect by irradiating the water protons coordinated to the Eu(III) ion which resonate at ca. 50 ppm downfield from bulk water at 312°K. Since this signal is extremely broad, it is again convenient to use a selective shape pulse in order to excite all the spins simultaneously. On this basis, a remarkable ST effect of ca. 85 % has been measured (train of 90° e-burp1 pulses of 1 ms each, total irradiation time 4s, irradiation power 660 Hz, pH 7.7, 30 mM, 312°K, 7.05T).

[0033] The ST effect shown by the lanthanide chelated complex according to the invention is further markedly sensitive to physico-chemical parameters of diagnostic interest wherein this allows their advantageous use in the method of the invention for the determination of said parameters either *in vivo* or *in vitro*.

[0034] A list of parameters of interest includes any physico-chemical parameter of diagnostic interest which is able to influence at least one factor which regulates the saturation transfer from the contrast agent and the surrounding water.

[0035] In particular said parameters include: temperature, pH, metabolite concentration, O_2 or CO_2 partial pressure, enzymatic activity, in a human or animal body organ or tissue.

[0036] According to the invention method the amount of saturation transfer is related to the physico-chemical parameter of diagnostic interest according to the following equation:

$$\left(1-\frac{M_s}{M_0}\right)=\left[\frac{k_{ex}n[C]}{2R_{1irr}[H_2O]+k_{ex}n[C]}(1-\exp[-(R_{1irr}+\frac{k_{ex}n[C]}{2[H_2O]})t])\right] \qquad [1]$$

[0037] According to this equation, the saturation transfer effect observed is conveniently quantified as $(1- M_S/M_0)$ wherein M_S refers to the intensity of the water signal upon irradiation at the frequency corresponding to the mobile protons resonance (ν^{on}) and M_0 indicates the water signal intensity measured upon irradiation at the frequency ν^{off} where $\nu^{off} = - \nu^{on}$ and $\nu^{water} = 0$.

[0038] The irradiation at ν^{off} allows to take into account the direct saturation effects on the water signal.

[0039] As indicated by equation 1, the ST effect is dependent on:

- the irradiation time, *t*;
- the pseudo first order kinetic constant rate of the irradiated protons k_{ex};
- the number of irradiated mobile protons *n*;
- the longitudinal relaxation rate of the bulk water upon irradiation of the mobile protons, R_{1irr};
- the molar concentration of the paramagnetic agent, *[C]* and of the bulk water protons, $[H_2O]$ (111.2 M in pure water).

[0040] One can safely assume that all the paramagnetic chelates preferred for the use in the method of the invention possess an exchangeable water molecule coordinated to the metal centre. For this reason, though R_{1irr} is conceptually different from R_1, it is likely that R_{1irr} is higher for paramagnetic systems than diamagnetic agent and, furthermore, it depends on the concentration of the metal complex.

[0041] Equation 1 indicates that the ST efficiency depends upon the concentration of the exchanging protons and therefore on the concentration of the contrast agent ($n[C]$). This finding makes the measurement of the diagnostic parameter of interest dependent on the contrast agent concentration. So, while no problems occur when the determination of said parameters is performed *in vitro*, where the concentration may be determined, an accurate *in vivo* determination thereof without knowing the local concentration of the administered agent is not possible.

[0042] The relationship between the concentration of the paramagnetic agent and the ST effect is not linear, unlike what is commonly observed for the relaxation enhancing ability of the conventional Gd(III)-based contrast agents. In fact, the steady-state value of the ST effect is determined by:

$$\left(1-\frac{M_s}{M_0}\right)=\frac{k_{ex}n[C]}{2R_{1irr}[H_2O]+k_{ex}n[C]} \qquad [2]$$

where it is evident that the dependence of the concentration of the contrast agent is less marked than in the conventional MRI contrast agents, even by taking into account the concentration dependence of R_{1irr}.

[0043] So, in spite of the limited role played by the concentration on the efficacy of a CEST contrast agent, the precise knowledge of the local concentration of the agent is still a necessary requisite for an accurate determination *in vivo* of the parameter of interest.

[0044] This problem may be solved by considering two pools of magnetically different labile protons whose ST effect has to show a different dependence from the physico-chemical parameter of interest.

[0045] Two strategies are possible:

i) the use of a paramagnetic contrast agent comprising a single CEST molecule endowed with both the two magnetically non equivalent labile protons pools; or

ii) the use of a paramagnetic contrast agent comprising two different molecules.

[0046] In the latter case the two molecules must have the same biodistribution pattern.

[0047] In a first preferred method according to the invention for the determination of physico-chemical parameters of diagnostic interest by use of CEST MR Imaging a single paramagnetic compound endowed with at least two magnetically non equivalent pools of mobile protons in exchange with the bulk water protons is used.

[0048] According to this method, the selective irradiation is performed on the two different pools of mobile protons. A ratiometric method is then exploited in order to remove the dependence of the ST effect from the absolute concentration of the administered contrast agent and to allow the determination of the physico-chemical parameter of interest both *in vitro* (*ex vivo*) and *in vivo* independently from the local concentration of the agent.

[0049] Preferably, the compound is a paramagnetic complex or a physiologically acceptable salt thereof. The metal ion is preferably selected among paramagnetic transition or Ln(III) metal ions on the basis of the ability to induce the ST effect through the involvement of two magnetically different sets of mobile protons belonging to the paramagnetic complex molecule. The chelating ligand may consist of any organic ligand endowed with at least two pools or, if one pool is represented by the water protons coordinated to the metal centre of the paramagnetic complex, at least one pool of mobile protons bound to a nitrogen, oxygen, sulphur, phosphorous atom. Preferably, the mobile protons pools belong the first to an amide group of the chelating ligand and the second to a metal coordinated water protons. More preferably, the mobile protons pools belong to the coordinating amide groups and to the metal bound water protons of the Eu(III) complex of the 1,4,7,10-tetraazaciclododecane-1,4,7,10-tetraacetic acid tetraglycineamide ligand (Eu-DOTAM-Gly).

[0050] In another preferred method according to the invention for the determination of physico-chemical parameters of diagnostic interest by use of CEST MR Imaging two paramagnetic compounds are used. According to this method, the selective irradiation is performed of two different pools of mobile protons which are provided by the two paramagnetic agents. A ratiometric method is again exploited which removes the dependence of the saturation transfer effect from the absolute concentration of the administered contrast agents. This method allows the determination of said chemical-physics parameter both *in vitro* (*ex vivo*) and *in vivo* independently from the local concentration of the agent but according to the known concentration ratio between the two agents.

[0051] Preferably, the two paramagnetic compounds are paramagnetic chelate complexes or a physiologically acceptable salt thereof in which the two chelated paramagnetic ions are different. Said two metal ions are preferably selected among paramagnetic Ln(III) ions on the basis of their ability to promote the saturation transfer effect through the involvement of two magnetically different exchanging proton pools. The two chelating ligands may consist of any organic ligand endowed with at least one mobile proton bound to a nitrogen, oxygen, sulphur, phosphorous atom and may be equal or different but suitably selected in order to grant the same biodistribution pattern for the two metal complexes. Preferably the mobile protons belong to an amide group and, more preferably, to the coordinating amide groups of a 1,4,7,10-tetraazaciclododecane-1,4,7,10-tetraacetic acid tetraglycineamide or of the 10-[(3-methoxyphenyl)methyl]-1,4,7,10-tetraazaciclododecane-1,4,7-tris-[(aminocarbonyl)methyl] chelating ligands.

[0052] In the most preferred method of the invention Yb(III)-DOTAM-Gly together with Eu(III)-DOTAM-Gly or a physiologically acceptable salt thereof are used. The first complex was chosen for the availability of chemically exchanging amide protons ($\Delta\delta$ = 16 ppm at 312°K) and the second one for the protons belonging to metal coordinated water molecule ($\Delta\delta$ of ca. 50 ppm at 312°K).

[0053] Equally preferred is the use of Tm(III)-DOTAM-Gly together with Eu(III)-DOTAM-Gly or a physiologically acceptable salt thereof.

[0054] Having the same electric charge, hydrophilic/lipophilic balance and analogous structure, the two metal complexes of both the couples are reasonably expected to show the same biodistribution pattern.

[0055] A further object of the invention is the use of one or more paramagnetic compound(s) comprising at least one mobile proton in chemical exchange with the water medium protons and able to generate a saturation transfer effect between said mobile proton and the water protons which is sensitive to the physico-chemical parameter of interest for the preparation of a pharmaceutical composition for the determination of said parameter in a human or animal body organ, fluid or tissue by use of CEST MR Imaging.

[0056] In an even further aspect the invention relates to a pharmaceutical composition which includes, together with a physiologically tolerable carrier, at least one paramagnetic compound characterized in that it comprises at least one mobile proton in chemical exchange with the water medium protons and it is able, when a proper radiofrequency rf irradiating field is applied at the resonance frequency of the said exchangeable proton, to generate a saturation transfer effect between said mobile proton and the water protons wherein said saturation transfer is related to the physico-chemical parameter of diagnostic interest.

[0057] This pharmaceutical composition allows the determination of a physico-chemical parameter of diagnostic interest selected from temperature, pH, metabolite concentration, O_2 or CO_2 partial pressure and enzymatic activity in a human or animal body organ or tissue by use of CEST Magnetic Resonance Imaging.

[0058] Preferably the pharmaceutical composition includes, together with a physiologically tolerable carrier, one paramagnetic chelated complex endowed with at least two magnetically non equivalent pools of mobile protons or a physiologically acceptable salt thereof wherein the composition including Eu(III)-DOTAM-Gly chelated complex or a physiologically acceptable salt thereof is more preferred.

[0059] Also preferably, the pharmaceutical composition includes, together with a physiologically tolerable carrier, two paramagnetic complex compounds. More preferably the composition includes two paramagnetic chelated complexes or a physiologically acceptable salt thereof whose two chelated paramagnetic metal ions are different and whose chelating ligands, which may consist of any organic ligand endowed with at least one mobile proton bound to a nitrogen, oxygen, sulphur, phosphorous atom, are selected in order to grant the same biodistribution pattern for the two metal complexes.

[0060] The two paramagnetic complexes are preferably comprised in equal molar amount or in a known molar ratio which is selected according to the two paramagnetic metal ions of the included complex compounds. Said ratio may range from 1 to 30, preferably it ranges from 1 to 10, more preferably from 1 to 5 and most preferably from 1 to 2, wherein the minimum molar concentration requested of the paramagnetic compound included in lower amount at least is 0.05 mM while the global concentration of the included paramagnetic CEST contrast agent ranges between 0.001 and 1.0 M.

[0061] Most preferably, the pharmaceutical composition comprises, together with a physiologically acceptable carrier, Yb(III) DOTAM-Gly an Eu(III) DOTAM-Gly or Tm-DOTAM-Gly and Eu-DOTAM-Gly or a physiologically acceptable salt thereof.

[0062] The pharmaceutical preparations according to the invention can be suitably injected intravasally (for instance intravenously, intraarterially, intraventricularly, and so on) or used by way of intrathecal, intraperitoneal, intralymphatic, intracavital, oral or enteral administration.

[0063] The injectable pharmaceutical formulations are typically prepared by dissolving the active ingredient(s) and the pharmaceutically acceptable excipients in water of suitable purity from the pharmacological point of view. The resulting formulation is suitably sterilised and can be use as such or it can alternatively be lyophilised and reconstructed before the use.

[0064] These formulations can be administered in concentrations depending on the diagnostic requirements, at a dose ranging from 0.01 to 0.5 mmol/kg body weight.

[0065] In order to test the validity of a preferred method according to the invention the CEST spectra of a solution containing 16 mM of Eu-DOTAM-Gly and 20 mM of Yb-DOTAM-Gly at pH 8.1 (7.05 T, 312°K, irradiation power 1050 Hz, irradiation time 4 s) were registered and the results are included in Figure 7. Interestingly, the detection of a "peak" (very broad) centred at about 50 ppm (downfield the water signal) is a clear indication of the saturation transfer occurring when the coordinated water protons of Eu-DOTAM-Gly are irradiated.

[0066] The ratiometric method on which are based the preferred methods according to the invention is derived from a re-arrangement of equation 1 and it is expressed by the following equation:

$$\frac{\left(\frac{M_0 - M_S}{M_S}\right)^A}{\left(\frac{M_0 - M_S}{M_S}\right)^B} = \frac{K^{conc} k_{ex}^A n^A R_{1irr}^B}{k_{ex}^B n^B R_{1irr}^A} \qquad [3]$$

where the superscripts A and B identify the paramagnetic complex compounds whose exchanging pools magnetic parameters are referred to. In the above experimentation, for example, A = Yb-DOTAM-Gly and B = Eu-DOTAM-Gly and K^{conc} which represents the [A]/[B] ratio is 1.25. The presence of two R_{1irr} values, one for each pool of labile proton irradiated, is due to the fact that, in principle, R_{1irr} depends on the exchange rate between the bulk water and the irradiated mobile protons which is different for the two proton pools.

[0067] The same equation holds also if one single compound with two pools of mobile protons is considered, but, in this case, K^{conc} is obviously equal to 1.

pH responsive CEST agents

[0068] Generally speaking, a good candidate as pH responsive CEST agent according to the method of the invention may be any paramagnetic complex compound which includes a Ln(III) metal ion and a chelating ligand which comprises at least one mobile proton whose chemical exchange with the water protons undergoes a basic or acid catalysis.

[0069] Moreover, any paramagnetic complex whose structure changes according to the pH in such a way to induce a chemical shift modification of the mobile proton(s) thereof can equally be used as pH responsive CEST agent according to the method of the invention.

[0070] Suitable pH responsive CEST agents further include all the paramagnetic complex compounds wherein the number of water molecules coordinated to the paramagnetic metal centre changes depending on the pH and a change of the relaxation rate of the bulk water protons occurs. As the pH dependence of the ST effect promoted by the mobile protons of amide groups thereon, the Ln(III) DOTAM-Gly complexes according to the invention represent a suitable class of pH responsive CEST agents.

[0071] The pH dependence of the ST effect has been assessed for the Yb-DOTAM-Gly derivative (30 mM, 312°K, irradiation power 1050 Hz, irradiation time 4 s) and the results is showed in Figure 5. The ST effect is markedly pH-dependent, being maximum at pH 8.1 and almost negligible at pH lower than 6. The pH dependence is linear (regression coefficient = 0.996) in the pH range 5.5 - 8.1, whereas at higher pH values the saturation transfer becomes less efficient likely because of the too extensive exchange broadening of the N-H resonances. This behaviour supports the hypothesis that the pH dependence of the ST effect mainly arises from the base-catalysed proton exchange of the amide N-H protons of the Yb(III) complex. Interestingly, similar result were obtained upon 2 s of irradiation. These results are very promising for an *in vivo* application of this chelate, since the ST effect is markedly pH sensitive and, moreover, it is properly tuned at the physiopathological pH interval.

[0072] An ^1H water MR image of a phantom containing a 30 mM solution of the agent at different pH values was further recorded at 7.05 T and 298°K on a Bruker Pharmascan imager. Interestingly, even at pH 5.4, where the directly measured CEST effect is quite low (12 %), the corresponding contrast in the image difference is not negligible at all.

[0073] According to the preferred method of the invention, the pH dependence of the CEST effect has been tested by using a mixture of two Ln(III)-DOTAM-Gly chelates differing in the lanthanide ion. Thus, Yb-DOTAM-Gly and Eu-DOTAM-Gly complexes were chosen in order to exploit the CEST effects associated with the exchange of the amide N-H protons and the metal coordinated water protons, respectively. In Figure 7 the CEST spectrum obtained from a solution containing 16 mM of Eu-DOTAM-Gly and 20 mM of YbDOTAM-Gly at pH 8.1 (B_0 = 7.05 T, 312°K, irradiation power 1050 Hz, irradiation time 4 s) is shown. Besides the peak due to the direct saturation of the bulk water, the CEST spectrum is characterized by two additional peaks: one, relatively narrow, is upfield shifted of ca. 16 ppm and the other, very broad, is downfield shifted at ca. 50 ppm from the chemical shift of the bulk water. Clearly, the first peak corresponds to the four amide N-H protons of the Yb(III) complex, whereas the second peak refers to the protons of the coordinated water in the Eu(III)-based chelate. According to the ratiometric method on which the method of the invention is based, the the CEST effect evaluated as $[(M_0-M_S)/M_S]_{YbL}/[(M_0-M_S)/M_S]_{EuL}$ ratio is not dependent on the absolute concentration of the contrast agents but only on their relative concentration ratio. On this basis, the pH dependence of the $[(M_0-M_S)/M_S]_{YbL}/[(M_0-M_S)/M_S]_{EuL}$ ratio was investigated at 7.05 T, 312 °K on a solution containing 16 mM of Eu-DOTAM-Gly and 20 mM of the Yb(III)-based chelate (irradiation time 4 s, irradiation power 1050 Hz). The results reported in Figure 9 show the high responsiveness to the pH of the system of the invention. Interestingly, the marked pH dependence observed for such system, which ensures a good sensitivity to the method, is basically due to the pH dependence of the ST effect shown by the Yb(III) chelate. In fact, the ST effect arising from the irradiation of the protons of the coordinated water in the Eu-DOTAM-Gly complex is basically unaffected in the investigated pH range from 5.5 to 8.5. This allows the full exploitation of the pH dependence of the Yb(III) chelate which results in a remarkable pH dependence of the ST ratio in pH range from 6.5 to 8.1 considerably larger than the one reported by Balaban and Ward in their diamagnetic system (Ward KM and Balaban RS. Magn Res Med 2000; 44: 799-802).

[0074] In another experiment we have checked the validity of one of the preferred method of the invention for the pH determination *in vitro* when a single paramagnetic complex endowed with two magnetically non equivalent pools of mobile protons is used. In Figure 10 the pH dependence of the ratiometric plot (amide protons over coordinated water protons) for a 30 mM solution of Eu-DOTAM-Gly at 312°K and 7.05 T is reported.

[0075] The experiment has been carried out by irradiating the two pools of mobile protons for 2 seconds with the same trains of selective e-burp1 shape pulses indicated above.

[0076] The data reported in Figure 10 suggest that the pH dependence is maintened, even if the sensitivity of this method is quite significantly reduced with respect to the data reported in Figure 8. The reason is due to the less marked pH dependence shown by the ST effect of the amide protons for Eu(III)-DOTAM-Gly complex.

Temperature responsive CEST agents

[0077] The exchanging rate of any mobile proton is temperature dependent.

[0078] The temperature can also affect the chemical shift value of the exchanging protons induced by the paramagnetic metal and the T_1 value of the water signal. On this basis any paramagnetic complex whose chelating ligand includes at least a mobile proton can advantageously be used as temperature responsive CEST agent according to the method of the invention.

[0079] In case of mixed valence compound (D.E. Richardson and H. Taube in Coord Chem Rev 1984, 60:107-129), the change of the electron spin configuration caused by a temperature variation can also be exploited for the attainment of a temperature responsive CEST agent according to the method of the invention.

[0080] The responsiveness to the temperature according to the method of the invention has been tested by use of a composition containing Tm(III)-DOTAM-Gly together with the Eu(III) complex of the same ligand. By irradiation of the amide protons of the Tm(III)-DOTAM-Gly and the protons of the water molecule coordinated to the Eu(III)-complex according to the method of the invention a satisfactory results, as shown in figure 11, as been obtained.

Metabolite concentration responsive CEST agents

[0081] In order to be responsive to the presence of a specific metabolite, a paramagnetic CEST agent has to be able to interact non covalently and as selectively as possible with it and this interaction must promote changes in the parameters determining the saturation transfer efficacy such as, for example, chemical shift, exchange rate, relaxation rate of bulk water, number of mobile protons.

[0082] The responsiveness to a given metabolite according to a preferred method of the invention has been tested *in vitro* by using the Yb(III) complex of the heptadentate 10-[(3-methoxyphenyl)methyl]-1,4,7,10-tetraazaciclododecane-1,4,7-tris-[(aminocarbonyl)methyl] chelating ligand. The chelating ligand of this paramagnetic complex has been prepared as disclosed in the European patent Application 01124440.7. This chelate owns 6 mobile amide protons whose chemical shift separation from the bulk water is ca 29 ppm upfield the bulk water signal at 312°K. This complex is able to interact quite strongly with several anionic substrate endowed with coordinating groups able to replace the water molecules coordinated to the metal centre.

[0083] Among the anionic substrates of interest for this application, one may include both endogenous and exogenous compounds.

[0084] More preferably, the endogenous substrates are selected from the group consisting of lactate, citrate, carbonate, phosphate, pyruvate, natural amino-acids, oxalate, tartrate, succinate, choline, creatine, acetate, and malonate.

[0085] Particularly preferred substrates are human metabolites, wherein lactate, citrate, carbonate, and phosphate are the most preferred.

[0086] Moreover, the substrate molecule of the invention can be an exogenous substance, wherein the term exogenous, as used herein, refers to any substance of pharmacological or diagnostic interest, eventually modified in order to allow a suitable binding to the paramagnetic complex.

[0087] As a representative, but not limiting, example, we have considered L-lactate.

[0088] The affinity constant between the metal complex and L-lactate has been evaluated through relaxometric measurements carried out on the Gd(III) complex (K_A of ca. 3000 at 298°K and pH 6.5). The exchange between the free and the lactate-bound Yb(III) complex is slow on the NMR frequency timescale. Therefore, different resonances for the mobile amide protons for the two forms ($\Delta\omega$ of 29 ppm and 15 ppm for the free- and bound-forms, respectively) of the metal complex may be detected at physiological conditions (312°K and pH 7.4) in the ^{1}H-NMR spectrum.

[0089] The signals corresponding to the two forms of the CEST agent are sufficiently separated to allow their selective irradiation. The dependence on the L-lactate concentration of the ST effect promoted by the irradiation of the free amide protons in a 9.3 mM solution of Yb(III) complex of the heptadentate 10-[(3-methoxyphenyl)methyl]-1,4,7,10-tetraazaciclododecane-1,4,7-tris-[(aminocarbonyl)methyl] chelating ligand is shown in Figure 12 (7.05 T, pH 7.4, 312K, irr. power 1050 Hz, irr. time 6s).

[0090] Interestingly, the ST efficiency shows a marked dependence in the range of Lactate concentration (0-10 mM) of diagnostic relevance.

BRIEF DESCRIPTION OF THE DRAWINGS

[0091]

Figure 1: ^{1}H-NMR spectra of Yb-DOTAM-Gly (7.05 T, 298 K and pH 7) in D_2O (top) and H_2O (bottom).

Figure 2: Chemical shift difference ($\Delta\omega$ in ppm) between the amide N-H protons and bulk water for Ln-DOTAM-Gly chelates. (7.05 T, 312K)

Figure 3: Dependence of the saturation transfer on the irradiation time (irradiation power 1050 Hz) for Dy-(30 mM, down-triangle), Ho-(30 mM, up-triangle), Er-(40 mM, diamond), Tm-(40 mM, circle) and Yb-(30 mM, square) chelates of DOTAM-Gly (B_0 = 7.05 T, 312 K, pH 8.1).

Figure 4: CEST spectrum of a 30 mM solution of Yb-DOTAM-Gly at pH 8.1 (B_0 =7.05 T, 312 K, irradiation time 4 s , irradiation power 1050 Hz).

Figure 5: pH dependence of the saturation transfer effect for a 30 mM solution of Yb-DOTAM-Gly (B_0 = 7.05 T,

312 K, irradiation power 1050 Hz, irradiation time 4 s).

Figure 6: 7.05 T Spin-echo image of a phantom containing 4 vials of Yb-DOTAM-Gly (30 mM) in the pH range 5.4-8.4. The vials were dipped in water containing 30 mM of Yb(III) aqua-ion (298 K, irradiation time 4 s). The image is the difference between two T1-weighted images (TR = 3.04 s; TE = 18.3 ms) in which the pre-saturation pulse was centred first on the amide protons (-4794 Hz from bulk water) and then symmetrically off-resonance (4794 Hz from bulk water).

Figure 7: CEST spectra of a solution containing 16 mM of Eu-DOTAM-Gly and 20 mM of Yb-DOTAM-Gly at pH 8.1 (B_0 = 7.05 T, 312 K, irradiation time 4s, irradiation power 1050 Hz).

Figure 8: pH dependence resulting by the exploitation of the ratiometric method (Eu-DOTA-Gly concentration is = 10 mM, Yb-DOTAM-Gly concentration is 12.5 mM; 7.05 T, 312 K, irradiation time 4s, irradiation power 1050 Hz). The error bars indicate the standard deviation of 5 independent measurements.

Figure 9: pH dependence of ST effect for a solution containing 10 mM of Eu-DOTAM-Gly and 12.5 mM of Yb-DOTAM-Gly (7.05 T, 312 K, irr. time 4s, irr. power 1050 Hz). The square refer to the irradiation of the amide protons of the Yb(III) complex and the circle correspond to the irradiation of the coordinated water protons in the Eu(III) chelate.

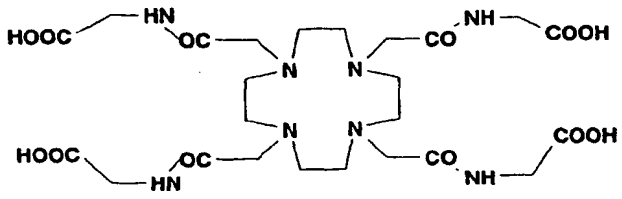
Figure 10: pH dependence resulting by the exploitation of the ratiometric method of a 30 mM solution of Eu-DOTAM-Gly (7.05 T, 312 K, irr. time 2s). The saturation has been performed by using a train of 270° e-burp1 pulse (20 ms each, power 50 Hz) for the amide protons and a train of 90° e-burp1 pulse (1 ms each, power 600 Hz) for the metal bound water protons.

Figure 11: Temperature dependence resulting by the exploitation of the ratiometric method (Eu-DOTAM-Gly = 14 mM; Tm-DOTAM-Gly = 14 mM; 7.05 T, pH 7.4, irr. time 4s, irr. power 1050 Hz).

Figure 12: Dependence on the L-lactate concentration of the ST effect of a 9.3 mM solution of Yb(III) complex of the 10-[(3-methoxyphenyl)methyl]-1,4,7,10-tetraazaciclododecane-1,4,7-tris-[(aminocarbonyl)methyl] chelating ligand measured by irradiating the amide protons of the free chelate at -29 ppm from the bulk water protons (7.05 T, 312 K, pH 7.4, irr. time 4s, irr. power 1050 Hz).

EXPERIMENTAL SECTION

[0092] Preparation of 1,4,7,10-tetraazaciclododecane-1,4,7,10-acetic acid tetraglycineamide (DOTAM-Gly):



[0093] The chelating ligand was synthesised according to the following steps:

a) exhaustive alkylation of TAZA (TAZA = 1,3,5,7-tetraazacyclododecane, 0.075 mol) with N-(2-Bromoethanoyl) ethyl glycinate (0.3 mol) in the presence of 0.3 mol of K_2CO_3 as base to give the corresponding tetraethyl ester derivative.

The reaction was carried out in acetonitrile by heating at 70°C for 6 h. After removal of the undissolved materials by filtration, the product was simply obtained by evaporating the solvent . Yeld: 91.4%

N-(2-Bromoethanoyl) ethyl glycinate was syntetisized according to the published procedure. (Kataki R, et al. J Chem Soc Perkin Trans 2 1992; 8:1347-1351).

b) controlled saponification of the tetraethyl ester and isolation of the desired tetracarboxylic acid.

[0094] The saponification of the tetraester was carried out in 200 ml of ethanol/water (1:1) solution at 60°C. NaOH 1 N (232 ml) was added to maintain the pH of the solution constant (pH 11) for almost 45' . The reaction was complete after 1 h heating. The resulting orange solution was cooled down and acidified at pH 2.2 with HCl. The DOTAM-Gly ligand was separated from such solution by liquid chromatography (solid-phase: Amberlite® XAD-1600; eluent: water). Yield: 88%. The ligand has been characterized by MALDI-TOF Mass Spectrometry (calc. for $C_{32}H_{40}N_8O_{12}$, 632.63 amu; found 633.55 (MH +)).

Synthesis of the Ln(III) complexes

[0095] The Ln(III)-DOTAM-Gly complexes were prepared by mixing equimolar amount (0.3 mmol) of the ligand and the corresponding Ln(III) chloride in 10 ml of water (room temperature, pH 8, 30'). The recovered chelate complexes have been characterized by means of their ^{1}H-NMR spectra. The recovered data were consistent with the expected structures

NMR methods

[0096] The high resolution work has been carried out on a Bruker Avance 300 spectrometer operating at 7.05 T. The saturation transfer experiments were carried out at 312°K by irradiating the sample with a continuous wave pre-saturation square pulse (power of 1050 Hz) or by using a proper train of e-burp1 selective pulses. Four scans and 4 dummy scans were used for all the experiments.

[0097] NMR imaging was performed using a 7.05 T Bruker PharmaScan having actively shielded gradients 300 mT/m) and running ParaVison 2.1.1 software. Standard PDW (proton density weighted images) were obtained using a SE (spin-echo) imaging sequence (using Hermite shaped 90° and 180° pulses). NMR image adopted parameters were (TR/TE/NE= 3.0s/18.3ms/1); FOV (Field Of View) 30x30 mm^2; slice thickness 2mm and image matrix 256x256 points. A 2.25 Watt square shaped saturation pulse was applied for 4 s in the pre-delay of the spin-echo sequence. Two images were acquired, one with saturation of the amide protons at - 4794 Hz from bulk water protons and the other with the rf irradiation offset at 4794 Hz.

Claims

1. A method for the determination, by MRI, of a physico-chemical parameter in a human or animal body organ, fluid or tissue, wherein a CEST contrast agent is employed whose saturation transfer capability is correlated to the physico-chemical parameter of interest and a CEST MR image responsive for said parameter is registered, said method being **characterized in that** the CEST contrast agent comprises a paramagnetic complex compound.

2. The method of claim 1 wherein the CEST contrast agent comprises at least two magnetically non equivalent pools of mobile protons.

3. The method of claim 2 wherein the CEST contrast agent comprises a single paramagnetic complex containing at least two magnetically non equivalent pools of mobile protons or a physiologically acceptable salt thereof.

4. The method of claim 2 wherein the CEST contrast agent comprises at least two paramagnetic complexes with different paramagnetic metal ions and chelating ligand with the same biodistribution or a physiologically acceptable salt thereof.

5. The method of claim 1 in which the determination is performed *in vitro* or *ex vivo*.

6. The method of claim 1 wherein the determination is performed *in vivo*.

7. The method of claim 1 wherein the parameter of interest is selected from temperature, pH, metabolite concentration, O_2 or CO_2 partial pressure and enzymatic activity.

8. The method of claim 4 wherein the two paramagnetic complex compounds are administered in a molar ratio ranging from 1 to 30.

9. The method of claim 1 wherein the paramagnetic ion in the paramagnetic CEST contrast agent is selected from the group consisting of iron (II) (high spin), iron (III), cobalt (II), copper (II), nickel (II), praseodymium(III), neodimium (III), dysprosium (III), erbium (III), terbium (III), holmium (III), thulium (III), ytterbium (III), and europium (III).

10. The method of claim 1 wherein the chelating ligand of the paramagnetic CEST contrast agent is an organic ligand containing at least one mobile proton bound to a nitrogen, oxygen, sulphur or phosphorous atom.

11. The method of claim 10 wherein the mobile proton is an amide proton.

12. The method of claim 11 wherein the mobile proton belongs to the amide groups of a 1,4,7,10-tetraazaciclododecane-1,4,7,10-acetic acid tetraglycineamide or of the 10-[(3-methoxyphenyl)methyl]-1,4,7,10-tetraazaciclododecane-1,4,7-tris-[(aminocarbonyl)methyl] tris-amide derivative.

13. The method of claim 2 wherein the CEST contrast agent comprises Eu(III) DOTAM-Gly optionally together with Yb(III) DOTAM-Gly or Tm(III)-DOTAM-Gly.

14. A pharmaceutical composition for use in the method of claim 1 which includes, together with a physiologically acceptable carrier, a CEST contrast agent comprising a paramagnetic complex compound.

15. The pharmaceutical composition of claim 14 wherein the CEST contrast agent contains a paramagnetic chelated complex comprising at least two magnetically non equivalent pools of mobile protons or two paramagnetic chelated complexes whose chelating ligands have the same biodistribution pattern and the two chelated paramagnetic metal ions are different or a physiologically acceptable salt thereof.

16. The pharmaceutical composition of claim 15 comprising two paramagnetic complexes in a molar ratio ranging from 1 to 30.

17. The pharmaceutical composition of claim 15 which comprises Eu(III)-DOTAM-Gly optionally together with Yb-DOTAM-Gly or Tm-DOTAM-Gly.

18. The use of one or more paramagnetic compound(s) comprising at least one mobile proton in chemical exchange with the water medium protons and able, when a proper radiofrequency rf irradiating field is applied at the resonance frequency of the said exchangeable proton, to generate a saturation transfer effect between said mobile proton and the water protons wherein said saturation transfer relates to the chemico-physical parameter of interest for the preparation of a pharmaceutical composition for the determination of said parameter in a human or animal body organ, fluid or tissue by use of CEST MRI.

19. A compound selected among: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid tetraglycineamide (DOTAM-Gly), a chelate complex of the (DOTAM-Gly) chelating ligand with a paramagnetic metal ion selected from the group consisting of: iron (II) (high spin), iron (III), cobalt (II), copper (II), nickel (II), praseodymium(III), neodimium (III), dysprosium (III), erbium (III), terbium (III), holmium (III), thulium (III), ytterbium (III), and europium (III), and the phisiologically acceptable salts thereof.

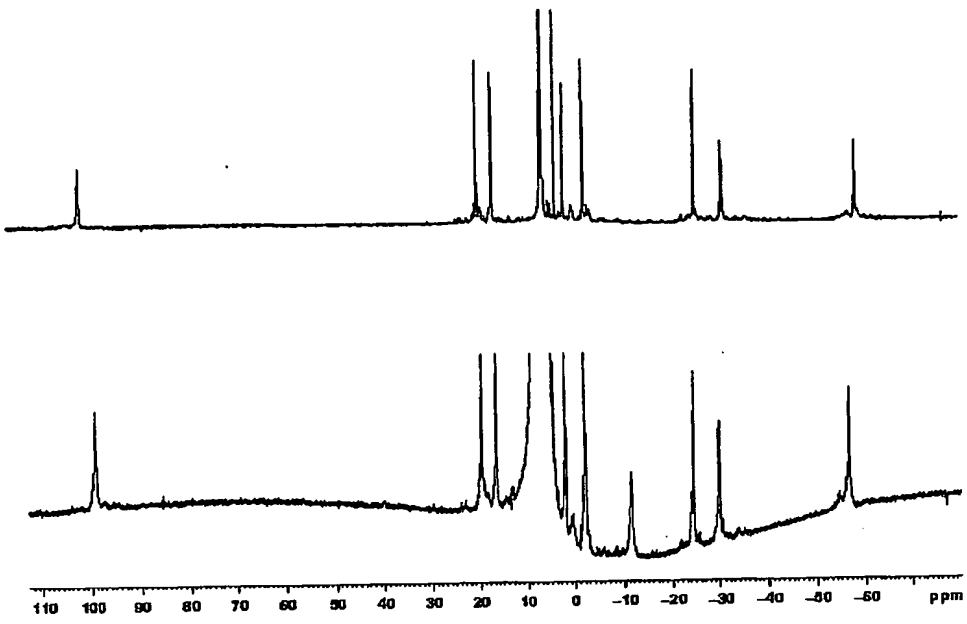


Figure 1

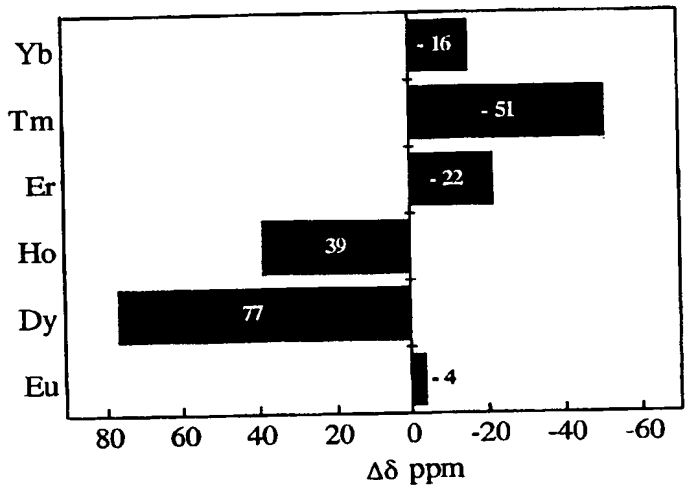


Figure 2

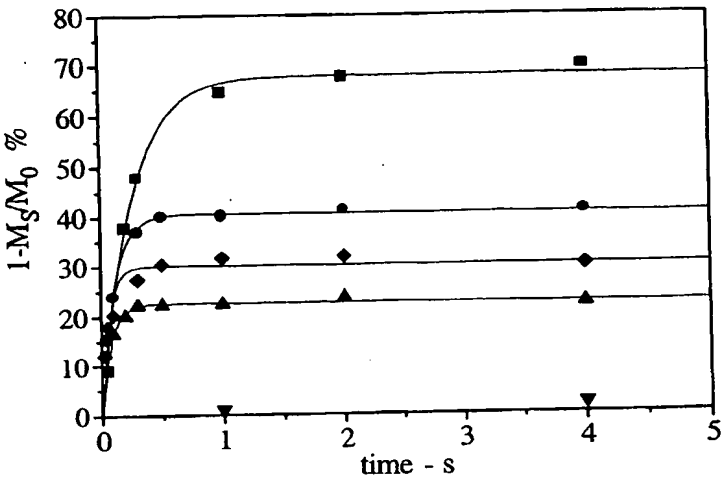


Figure 3

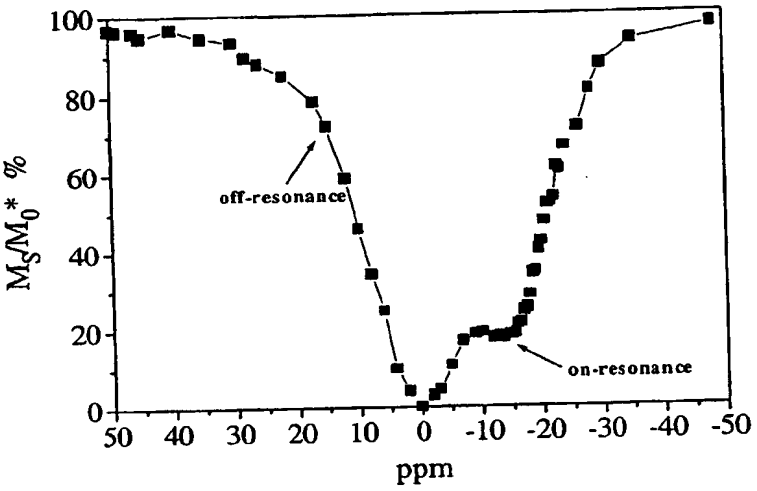


Figure 4

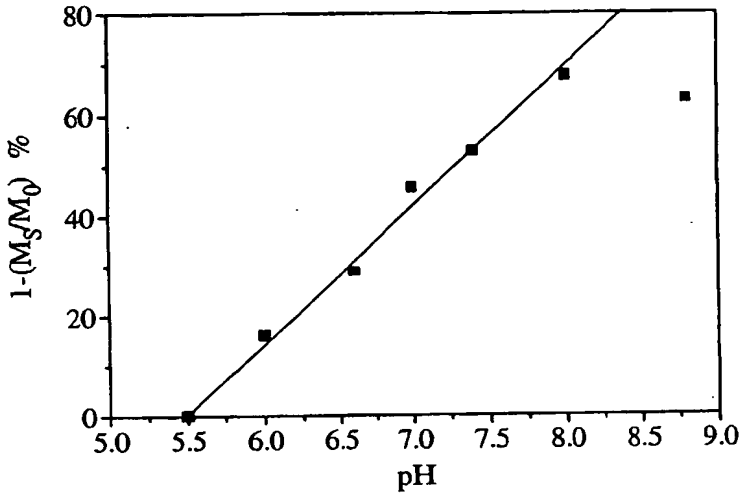


Figure 5

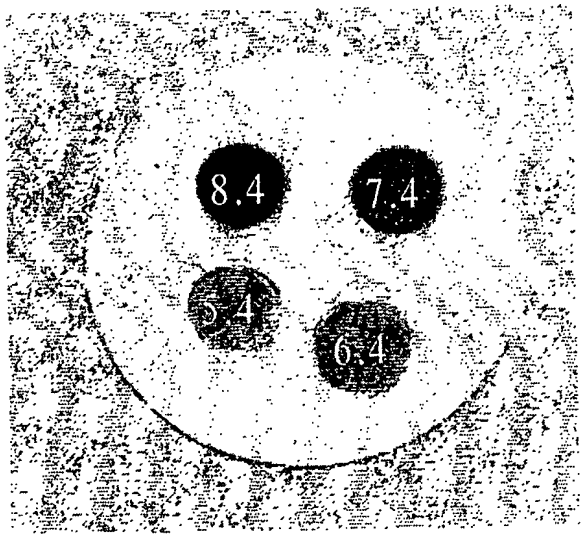


Figure 6

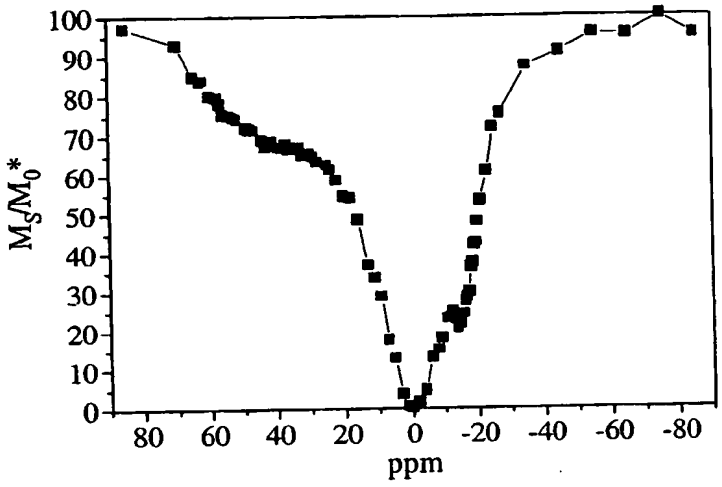


Figure 7

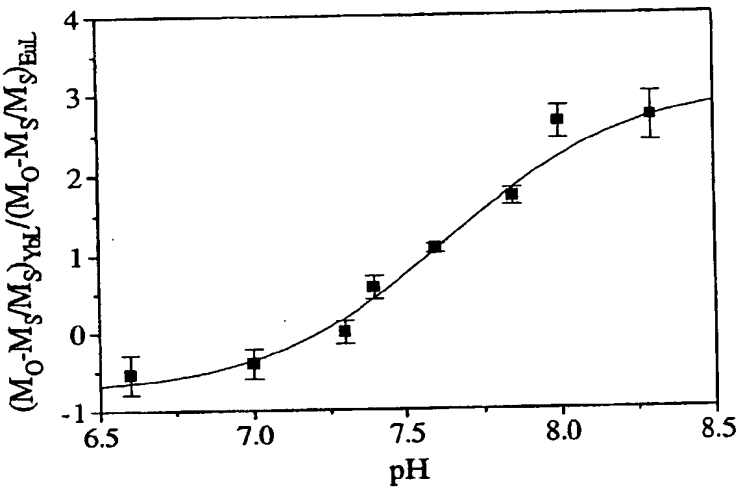


Figure 8

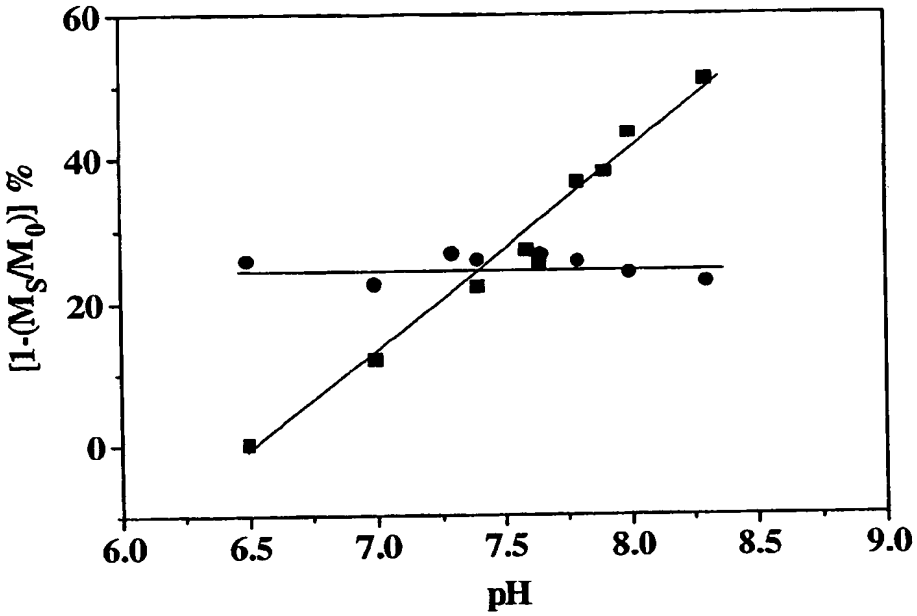


Figure 9

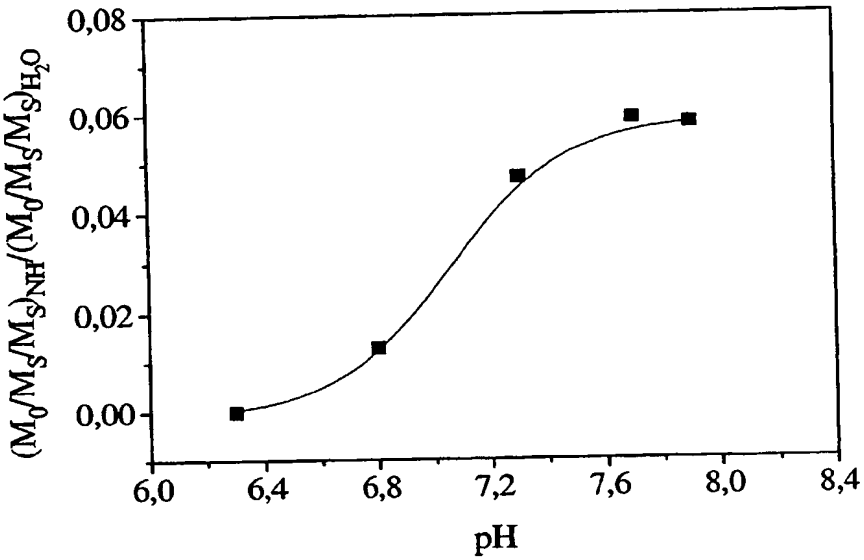


Figure 10

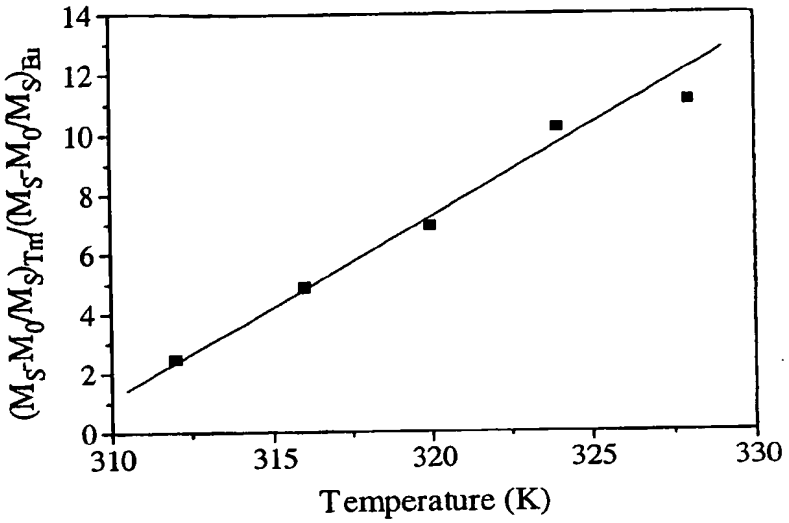


Figure 11

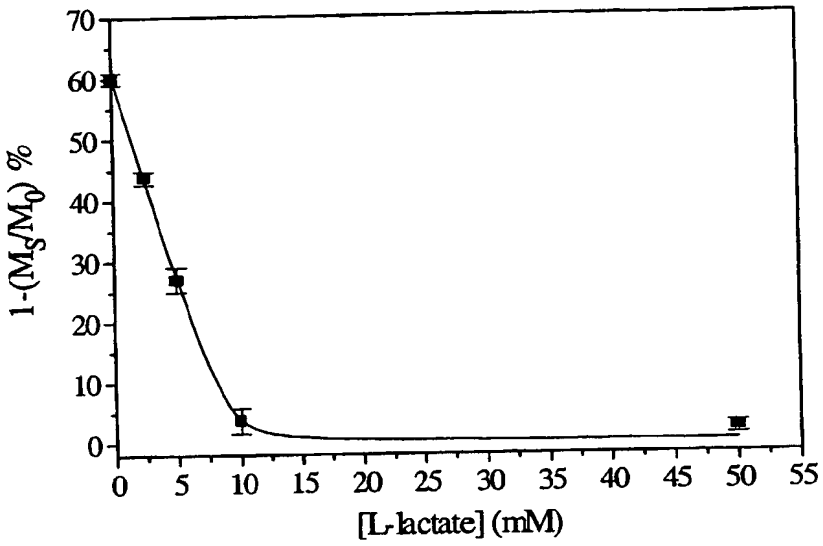


Figure 12

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 02 00 1178

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WARD K M ET AL: "A NEW CLASS OF CONTRAST AGENTS FOR MRI BASED ON PROTON CHEMICAL EXCHANGE DEPENDENT SATURATION TRANSFER (CEST)" JOURNAL OF MAGNETIC RESONANCE, ACADEMIC PRESS, ORLANDO, FL, US, vol. 143, no. 1, 2000, pages 79-87, XP000972579 ISSN: 1090-7807 * abstract * * figure 1 * * table 1 * * page 87, left-hand column, last paragraph * ---	1-8,10, 11, 14-16,18	A61K49/06
X	US 5 190 744 A (MOSELEY MICHAEL E ET AL) 2 March 1993 (1993-03-02) * abstract * * column 1, line 57 - column 2, line 4 * * column 4, line 40 - line 58 * * column 2, line 33-38,51-55 * ---	1-8,10, 11, 14-16,18	TECHNICAL FIELDS SEARCHED (Int.Cl.7) A61K
X	WARD K M ET AL: "DETERMINATION OF PH USING WATER PROTONS AND CHEMICAL EXCHANGE DEPENDENT SATURATION TRANSFER (CEST)" MAGNETIC RESONANCE IN MEDICINE, ACADEMIC PRESS, DULUTH, MN, US, vol. 44, no. 5, November 2000 (2000-11), pages 799-802, XP000969765 ISSN: 0740-3194 * abstract * * page 799, left-hand column, line 1 - line 17 * * page 801, left-hand column, last paragraph * --- -/--	1-8,10, 11, 14-16,18	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	19 March 2002	Villa Riva, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 02 00 1178

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
D,X	WO 00 66180 A (ALETRAS ANTHONY H ;US HEALTH (US); WARD KATHLEEN M (US); BALABAN R) 9 November 2000 (2000-11-09) * the whole document *	1-8,10, 11, 14-16,18	
Y	ZHANG S ET AL: "A NOVEL PH-SENSITIVE MRI CONTRAST AGENT" ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, VERLAG CHEMIE. WEINHEIM, DE, vol. 38, no. 21, 2 November 1999 (1999-11-02), pages 3192-3194, XP000864995 ISSN: 0570-0833 * page 3192, left-hand column, line 1 - line 3 * * figure 1 * * page 3193, right-hand column, line 43 - line 46 *	1-19	
Y	BALABAN R S ET AL: "MAGNETIZATION TRANSFER CONTRAST IN MAGNETIC RESONANCE IMAGING" MAGNETIC RESONANCE QUARTERLY, NEW YORK,NY, US, vol. 8, no. 2, 1992, pages 116-137, XP000982000 * abstract * * figures 1,2 * * applications p. 128-135 *	1-19	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	19 March 2002	Villa Riva, A

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D : document cited in the application
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EPO FORM 1503 03.82 (P04C01)

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EUROPEAN SEARCH REPORT

Application Number
EP 02 00 1178

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	AIME S ET AL: "High-resolution NMR and relaxometric studies of Ln(III) complexes of relevance to MRI" JOURNAL OF ALLOYS AND COMPOUNDS, ELSEVIER SEQUOIA, LAUSANNE, CH, vol. 225, 15 July 1995 (1995-07-15), pages 274-278, XP004072071 ISSN: 0925-8388 * the whole document *	1-19	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	19 March 2002	Villa Riva, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
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19-03-2002

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5190744 A	02-03-1993	AT 134450 T	15-03-1996
		AU 650009 B2	09-06-1994
		AU 7475391 A	10-10-1991
		CA 2077760 A1	10-09-1991
		DE 69117285 D1	28-03-1996
		DE 69117285 T2	31-10-1996
		WO 9114186 A1	19-09-1991
		EP 0518985 A1	23-12-1992
		ES 2085989 T3	16-06-1996
		HK 1001335 A1	12-06-1998
		IE 910777 A1	11-09-1991
		US 5494655 A	27-02-1996
		US 5833947 A	10-11-1998
WO 0066180 A	09-11-2000	AU 4656400 A	17-11-2000
		EP 1178837 A2	13-02-2002
		WO 0066180 A2	09-11-2000

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